Cotton (*Gossypium* spp.) pest management in the era of next-generation sequencing: A review

Julie Rebecca Joseph Mathari, Habeeb Shaik Mohideen*

Bioinformatics and Entomoinformatics Lab, Department of Genetic Engineering, College of Engineering and Technology, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu, Chennai, Tamilnadu-603203, India.

ABSTRACT

Cotton, as a cash crop, has huge economic importance. The high prevalence of pests, illnesses, weed pressure, the evolution of herbicide-resistant weeds, salinity, soil degradation, and climate anomalies such as droughts, floods, and heatwaves all limit cotton output around the world. Strategies like integrated pest management are employed to control the pest population across major cotton-producing countries. This method is effective as it aids in pest management and saves farmers a significant amount of money on pesticide purchases. Biotechnological advances have led to the development of Bt crops, which underwent a series of modifications according to the needs of farmers. Pyramid Bt and the effectiveness of RNA interference technology have been highlighted here. Also advances in the field of genomics have helped us understand plant-pest interaction. The following review is an overview of advancements in the field of cotton pests' management and the role of genomics and other bioinformatics approaches to better understand the effective management of pests with the least effect on the ecosystem. There is a rising need to develop methods to achieve Sustainable Development Goals (SDGs) aimed at sustainable development. Developments in the field of cotton research for SDGs have also been discussed here.

ARTICLE HIGHLIGHTS

The article discusses the importance of cotton crops and the various strategies employed to control the pest population. It also highlights the development of IPM strategies and how advancements in sequencing technologies have changed the course of pesticide design and development. It also discusses the need to identify novel targets that do not exhibit any off-target reactions.

1. INTRODUCTION

Cotton, referred to as "white gold" [1], is an important source of natural fiber, grown in around 80 countries. According to recent studies, India is the largest producer of cotton; it approximately produces 6.1 million tons per year. It contributes significantly to the economies of many developing nations, especially several West African countries [2,3]. For economically backward countries such as Burkina Faso, Mali, and Chad, cotton contributes to around 30–44% of the GDP [4]. Similarly, 12–32% is the contribution to GDP of developing countries like Uzbekistan, Tajikistan, and Turkmenistan, whereas it is approximately 4% in India [5]. Notably, 55% of the foreign exchange capital of Pakistan is attributed to cotton [6].

Around 18% of the overall GDP of developed countries is contributed by cotton [7,8].

Figure 1 gives an idea of the major cotton-producing countries across the world. The front runners in the cotton market are the following countries: India, the USA, China, Brazil, and Pakistan. The figure portrays the contribution of each country to world cotton production.

The most cultivated variety of cotton is *Gossypium hirsutum,* which is also referred to as upland cotton. *G. hirsutum* contributes to 95% of the total cotton production [11]. Successful cotton production requires 160 days above 15°C and a minimum of 50 cm of water [12]. The cotton crop faces many challenges, right from sowing to harvesting. The problems that it faces include insect pest infestation, diseases, heat, drought, cold, and salinity stresses, trash during picking, and post-harvest management problems [13]. These challenges cause a considerable amount of damage to the cotton yield. An extensive study of each problem is needed to understand it. This article presents a combined view of the losses incurred due to insect pest infestations and the relevant strategies to curb this menace. We have also shed light on the latest contribution of genomic technologies that could be utilized in pest management strategies.

2. EFFECT OF PESTS ON COTTON PRODUCTION

Pest infestation and pathogens contribute to around 10–30% losses in cotton crop production [14]. The cotton crop is affected by approximately

Corresponding Author:
Habeeb Shaik Mohideen,
Department of Genetic Engineering College of Engineering and Technology,
SRM Institute of Science,Chennai, Tamilnadu-603203, India.
E-mail: habeeb_skm @ yahoo.co.in

1300 arthropods worldwide; however, only 100 species of insects and mites may cause severe damage if left uncontrolled [15,16]. Several new strategies in pest control have emerged that can be a potential alternative to current approaches to regulating insect pests, nematodes, and pathogens.

About 80–90% [17] of the diseases target the leaves. Cotton leaf curl viral disease [18] is a predominant disease that affects the leaves. Resistance to pesticides has become a greater threat to pest control [19-21]. The crop is susceptible to a wide range of pests, including Lepidopteran larvae as well as sap-feeding hemipterans such as mirids, whiteflies, and aphids [22].

Bollworms, foliage feeders, and sucking pests are among the most destructive pests that lower yields in cotton crops. Among these pests, the bollworms such as *Helicoverpa armigera*, *Earias vitella*, *Earias insulana*, and *Pectinophora gossypiella* affect non-transgenic cotton globally, whereas the sucking pests - *Amrasca bigutulla*, *Bemisia tabaci*, and *Aphis gossypii* have started posing a threat to transgenic cotton besides their regular appearance on non-transgenic cotton as well [23]. The whitefly and PBW are exotic invaders. Even though the levels of crop losses due to pests appear to be small, their economic impact can be significant [24]. The major pests in the USA include species like aphids, whiteflies, plant bugs, tobacco budworms, bollworms, beet and fall armyworms, spider mites, and stinkbugs [25]. In Brazil, pests like cotton boll weevil and *H. armigera* affect cotton crops the most [26].

3. PEST MANAGEMENT STRATEGIES

The various strategies to manage these pests include: Cultural controls are practices that reduce pest establishment, reproduction, dispersal, and survival. Biological control, or biocontrol, is a method of controlling pests such as insects, mites, weeds, and plant diseases using other organisms. Behavioral controls utilize some chemicals to modify insect pest behavior and control pests without the use of toxins. Mechanical pest control is the management and control of pests using physical means such as fences, barriers, or electronic wires. Microbial control of insects is the concerted use of insect-specific pathogens and nematodes for the biological control of insects [27]. Natural enemies have been found in cotton systems in around 500 species, although only a few have been studied. 76 species of important arthropod predators and parasitoids of lepidopteran pests, belonging to 53 genera, and 46 species of natural enemies of sucking pests, belonging
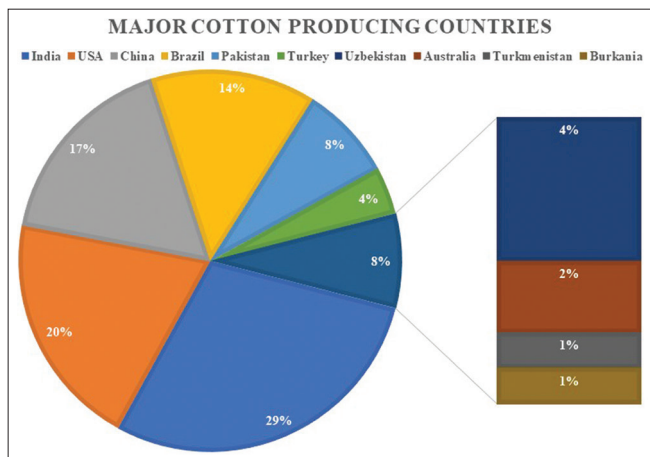

Figure 1: A pictorial representation of the major crop producing countries across the world. [9,10]

to 29 genera, have been described [28]. In India, crop rotation with sorghum, for example, is a strategy that the Central Institute for Cotton Research (CICR) has successfully proven so far. Selection of sucking pest-tolerant cultivars, synchronized sowing with correct spacing for the soil type, paired row planting irrigation, balanced application of organic and inorganic nutrients, intercropping with crops like cowpea, green gram, foxtail millet, or onion, avoidance of intercrops like bhendi, tomato, brinjal, and castor bean, removal of weeds with clean cultivation, and discouragement of monocropping [29].

An ideal pest management strategy must be environmentally, economically, and farmer-friendly. Silent Spring, written by Rachel Carson in 1962, called the public's and scientists' attention to the issues caused by pesticides. Silent Spring also drew the scientific community's attention to the negative externalities of pesticide use [30]. Due to the indiscriminate use of pesticides, there is a ready development of resistance against them [31]. Chemical-based management with various groups of active compounds is gradually losing effectiveness against major pests; this has given rise to new strategies of pest management called integrated pest management (IPM) [32]. There have also been advances in the fields of genomics and biotechnological technologies, which have further paved the way for a new generation of pest management strategies.

The various pest management strategies followed by major cotton-producing countries around the world are listed in Table 1.

4. IPM

Smith and van dan Bosch (1967) coined the term "Integrated Pest Management," which was formally recognized by the United States National Academy of Sciences in 1969 [41]. The IPM strategies include the judicious use of synthetic organic pesticides, the reintroduction of beneficial insects, crop diversification schemes, the planting of early-maturing varieties, and the destruction of cotton crop residues [42]. Emphasis has been given to the management of pests by using IPM strategies in Pakistan. The major cotton-growing countries are now shifting to this method of pest management because of its feasibility and because it causes lowered stress on the ecological balance. The cotton growers in Canete Valley, Peru, were the first growers to understand the effect of combining pest management strategies since they observed it as being effective in managing pests. The concept of IPM, however, was adopted by the USA in 1959. It substantially became an effective approach in various parts of the world, like Europe, Asia, Australia, Latin America, and Africa. Various governments have passed laws to encourage and implement IPM strategies for the eradication of various pest populations, like the Bollworm Eradication Program in the USA. The UN Conference on Environment and Development (Rio de Janeiro, Brazil) also validated the ecological basis of the concept of IPM [23].

IPM has been an important strategy in the control of pests as it involves the combination of different types of methods of pest management. In India, many IPM programs have been implemented and proposed. These programs were initially proposed to reduce the application of insecticides. The very first program in IPM was conducted under the operational research program. There is a need to educate the farmers about the knowledge and analytical skills required for using the strategies of IPM [43]. The implementation of IPM is as long as six decades since it was first introduced [49]. It has been four decades since it was implemented in the USA [44], Asia [45], India [46], Latin America [47], and Australia [42]. However, in economically backward countries, it is still not fully accepted.

Table 1: Pest management strategies by leading cotton-producing countries across the world.

Country	Pest	Management
USA [33]	Boll weevil	Pheromone traps, diflubenzuron at pinhead square stage, Boll weevil eradication program
	Bollworms	DDT sprays with toxaphene [34], low doses of methyl parathion [35]
	Pink bollworms	Growing Bt suppressed population in the first half and using sterile insect techniques (SIT) Bollworm Eradication program has been launched by the US government for its complete eradication.
	Tobacco bollworm	Plant-incorporated protectant (PIP)
	Nematodes	Using peanut as a rotational crop
	Aphids	Combination of natural predators and pesticides
India [36]	Pink bollworm	Integrated pest management (monitoring, mass trapping, mating disruption)
	Leafhopper	Pesticides like Azadirachtin, Acephate, Acetamiprid, Imidacloprid, Endosulfan, Cypermethrin, parasitoids *Stethynium triclavatum* and natural predators of Lady beetle
	Whitefly	Using yellow sticky traps, insecticides Azadirachtin, Acetamiprid, Buprofezin, and parasitoids *Encarsia* spp. and predators *Dicyphus hesperus*
	Thrips	Predatory mites (*Amblyseius swirskii*), insecticides Buprofezin, Carbaryl, Diafenthiuron
	Aphids	Green lacewing *Chrysoperla zastrowi*, insecticides like Azadirachtin, Acetamiprid, Dinotefuran, Fipronil
	Mealybugs	Parasitoids like *Aenasius bambawalei*, predators like Predatory wasps
	Tobacco caterpillar	Parasitoids like *Trichogramma chilonis, Chrysoperla carnea*, insecticides Chlorantraniliprole, Chlorfluazuron, Cypermethrin, Bt cotton
	Spotted and spiny bollworm	Yellow sticky traps, insecticides like Azadirachtin, Parasitoids *Aphelinus* spp*., Erythmelus empoascae*, predators *Chrysoperla carnea*, Bt
	Helicoverpa bollworm	Insecticides like Azadirachtin, Parasitoids like *Campoletis chloridae*, Entomopathogenic *Nomuraea rileyi*, and predators *Chrysoperla carnea*
	Leaf roller	Parasitoids like *Xanthopimpla punctata, Brachymeria* spp. n. euploeae
	Red cotton bug	Insecticide Fluvalinate
	Dusky cotton bug	Insecticides Spintoram, Acetamiprid, Pyriproxifin, Profenofos
	Stem Weevil	Insecticide carbofuran, Carbaryl
	Shoot weevil	Use of trap/border crops like okra, cannabis, castor, marigold, early pigeon pea, jowar, and maize crops is recommended
China [40]	Cotton bollworm	Natural predators like *Trichogramma* spp., *Orius similis*, and *Microplitis mediator*, Irrigation in winter and early spring can kill most pupae, Bt variety Cry1Ab
	Aphids	Intercropping cotton with wheat, Insecticide-treated seed mainly synthetic pyrethroids and organophosphate insecticides, planting Bt crops
	Mirids	Pesticides, and mirids are resistant to Bt crops
	Spider mites	Acaricides, weed management
	Pink bollworm	Monitored daily using light traps and pheromone traps, Bt variety Cry1Ac and Cry1(c)
Brazil [41]	Pink bollworm	Area-wide uniform planting dates, a cotton-free period, early and uniform stalk destruction, Cultivar manipulation
	Cotton stem borer	
	Aphis gossypii	Natural predators *Chrysopa externa, Cyeloneda sanguinea, Pseudodoros clavatus*
	Eutinobothrus brasiliensis	Area-wide uniform planting dates, Cotton-free periods, destruction of infested squares, bolls, Early and uniform stalk destruction, use of trap crops, and crop rotation
	Frankliniella spp.	
	Alabama argillaeea	
	Anthonomus grandis	Cultivar manipulation which includes rapid fruiting and early maturity, use of pheromones in along with pathogens
	Heliothis virescens	Bt protein Cry1Ac, pesticides like spiromesifen, bifenthrin, carbosulfan
	Tetranyehus (T.) urticae	Pesticides like spiromesifen, bifenthrin, carbosulfan
	Spodoptera litura	*Bacillus thuringiensis*, the use of pathogenic bacteria in combination with the female sex pheromone
	Helicoverpa armigera	Monitoring plants for eggs and young larvae and natural enemies that could be damaged by chemicals; *Bacillus thuringiensis* or control insects on organically grown plants; appropriate chemical treatment may be required for control in the commercial plantations.
Pakistan	*Scirotothrip dorsalis*	Insecticides like acephate, imidacloprid, thiamethoxam, lambda-cyhalothrin, buprofezin, fipronil [37]
	Amrasca devastans	Insecticides like acetamiprid, imidacloprid, thiamethoxam [38]
	Bemisia tabaci	Insecticides like acephate, fenpropathrin, lambda-cyhalothrin, and bifenthrin [39]
	Earias vittella	Insecticides like pyrethroids (cypermethrin, zeta-cypermethrin, deltamethrin, esfenvalerate, bifenthrin, lambda-cyhalothrin), organophosphates (chlorpyrifos, profenofos, triazophos, phoxim) [40]

5. THE EMERGENCE OF BT COTTON

Insect-resistant varieties of the cotton cultivar Bt cotton were introduced first in 1996 [48]. *Bacillus thuringiensis* is resistant to many bollworms and has been commercially cultivated in many countries, like the USA, Australia, China, Mexico, Argentina, South Africa, and India. Bt cotton has proven to be an effective control against the major pests of cotton in China and India. Farmers may be able to reclaim the economic and environmental gains of previous years by combining Bt-cotton seed with other forms of biological pest control [49]. Bt cotton was the first genetically modified crop introduced in India. Bollgard cotton (a trademark given to crops producing Bt against bollworm) is cultivated in more than one-third of the USA [50]. In China, it has reduced the economic burden on farmers by reducing the cost of insecticides to control pest populations [51]. A new approach to control the pest population involves the use of pyramid crops that produce more than one Bt toxin to control pest resistance. This approach has been widely used to delay the resistance of pests to a certain toxin, and studies have shown the efficiency of this method. The pyramid Bt crop becomes available in the US after 6 years of the commercialization of Bt cotton [52]. The primary threat to the Bt's success is the evolution of resistance to the pests [53]. There have been instances in which RNA interference (RNAi) and Bt toxins have been used to produce transgenic crops resistant to *H. armigera.* The crop produces double-stranded RNA to tackle the pest's metabolism of juvenile hormones [54]. The release of sterile moths in cotton fields has been reported to have reduced the population of pink bollworm, *P. gossypiella,* to about >99% without almost no insecticide application against this invasive pest [55]. A study stated that the development of resistance in the pink bollworm may have been caused by the scarcity of refuge in countries like India and China. Another reason could be the fact that the Bt toxin Cry1Ac is produced in lower concentrations in the crops produced in India and China [56].

6. GENOMIC APPROACHES TOWARDS THE MANAGEMENT OF PESTS

Sanger sequencing is considered the 'gold standard for nucleic acid sequencing and played an important role in the Human Genome Project [57]. However, it has a few drawbacks, including a high sequencing cost and being tedious [58]. The capabilities of high-throughput sequencing methods are used in RNA sequencing (RNA-Seq) to provide insight into a cell's transcriptome. It also provides significantly more coverage compared to Sanger sequencing and microarray-based methods. It provides greater resolution and higher coverage for understanding the transcriptome of an organism [59]. Next-generation technologies have paved the way to a deeper and better understanding of plant and insect interactions. The transcriptome data can be used to evaluate the gene expression profile [60]. The speed and accuracy of these technologies have been employed to identify and detect microRNAs, 3' and 5' untranslated regions, and even complete mRNAs [61,62].

Second-generation sequencing techniques have been widely used in the healthcare sector for the discovery of new targets and therapeutics that may suppress the expression of diseased genes [63]. Several studies have indicated that genomic approaches provide unbiased results for the discovery of complex mechanisms associated with different diseased conditions [64]. The noncoding parts of the genes have been explored to discover novel drug targets and the possible mechanisms for a particular abnormality [65]. High-throughput sequencing and RNA-Seq have helped us to investigate species that are not established genetic models yet display adaptations that make

them closely related to an array of ecological and physiological questions [66]. In the field of agricultural sciences, genomics has played a major role in unraveling various novel genes and targets that can reduce the off-target effects of many pesticides. A new field of ecological genomics has emerged as a result of these advancements. Ecological genomics combines high-throughput sequencing and novel analysis, which are within the scope of fundamental and applied research [67]. Next-generation sequencing has enabled us to get massive amounts of data to look beyond the model organisms for virtually any organism, which includes agriculturally important pests and associated microbial communities [68]. It has also helped in developing RNAi machinery for the control of these voracious pests. RNAi machinery has been developed for Asian corn borer *Ostrinia furnalalis* [69] and *H. armigera* [70]. RNAi has proven to be an important reverse tool for studying gene function. RNAi is a post-transcriptional gene silencing process in which the double-stranded RNAs suppress the expression of homologous endogenous genes by activating the degradation of mRNAs [71].

The cotton crop is affected by a wide range of arthropods; hence, it has been extensively studied, and it is considered a good model for studying various interactions between pests and crop interactions as well as different biological processes [72]. It has also served as a model to understand metal pollution in soil [73] and salt stress [74]. Cotton has a defense mechanism where it produces secondary metabolites like gossypol to inhibit the viability of pests [75]. Transcriptome sequencing has opened a new arena of cotton research where it is now possible to understand and design strategies to control pest populations more precisely. This approach has widened the horizons of cotton research. There have been numerous studies on the transcriptome of cotton. The cotton plant undergoes biotic, abiotic, and enzymatic stresses when infested [76]. The infestation by cotton boll weevils has been shown to have altered the regulation of enzymes related to the kinase cascade, transcription factors, and reactive oxygen species [74]. The transcriptome profiling of cotton in response to *H. armigera* [75,76], *Aphis gosypii* [77,78], *Anthonomus grandis* [66,79], winged and wingless *A. gosypii* [80], *B. tabaci* [81], and cotton infested by bollworm [82] has given significant insights by unraveling the various genes involved in the defense mechanism. Novel targets like the ecdysone receptors [83], gene signatures in *Verticillium* wilt-resistant cotton to curb the aflatoxins produced by a group of fungi belonging to *Aspergillus* [90], and novel microRNAs were identified in another study [84]. The resistance mechanism of pests to pesticides has also been studied, which revealed that the resistance of the species to pesticides was a polygenic effect [85]. The abundance of microbiota in a major pest of cotton *H. armigera,* was studied, which can help us further understand the defense mechanism of these pests against the secondary metabolites of cotton [86]. Metagenomic analysis, which is a powerful approach to understanding the microbiota in the gut of any organism, was done to understand the microbiota in the whiteflies [87]. Molecular markers were identified in *Spodoptera frugiperda,* which caused resistance in the species [88]. The resistance mechanism in *B. tabaci* was studied using pyrosequencing [89]. Detoxification genes in the pest *Spodoptera littoralis* (Boisd.), which are acetylcholinesterase, cytochrome p450, and glutathione S-transferase, were identified [90]. These studies can be useful in understanding the mechanism of defense against the major pests of cotton. Sequencing technologies have made it possible to design control measures to curb pest populations. Once the target has been identified, it will make the task of identifying inhibiting compounds possible. The goal of identifying 'designer pesticides can become a reality if we can extract important and relevant information from the genomes of these pests.
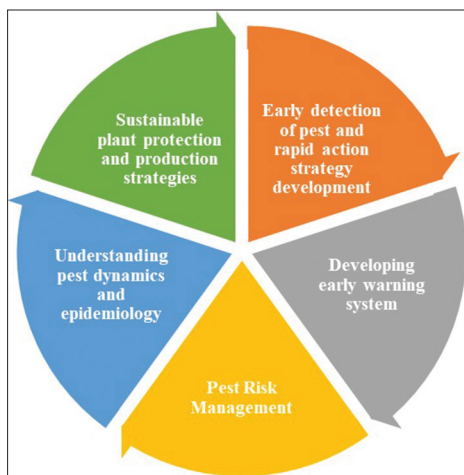
Figure 2: Illustration of the points to be considered for a sustainable management of pests.

7. SUSTAINABLE DEVELOPMENT GOALS IN COTTON PRODUCTION

The textile industry is marching toward cutting down its dependency on fossil fuels and is encouraging research that works along the lines of the SDGs. 17 SDGs were installed by 193 countries in 2015 [91]. The second SDG is aimed at achieving global food security and agricultural sustainability [99]. The 17 SDGs are as follows [92]:

1. SDG 1: No poverty
2. SDG 2: Zero Hunger
3. SDG 3: Good health and well-being
4. SDG 4: Quality Education
5. SDG 5: Gender equality
6. SDG 6: Clean water and sanitation
7. SDG 7: Affordable and clean energy
8. SDG 8: Decent work and economic growth
9. SDG 9: Industry, innovation, and infrastructure
10. SDG 10: Reduced inequalities
11. SDG 11: Sustainable cities and communities
12. SDG 12: Responsible consumption and production
13. SDG 13: Climate action
14. SDG 14: Life below water
15. SDG 15: Life on land
16. SDG 16: Peace, justice, and strong institutions
17. SDG 17: Partnerships for the Goals.

The NGS technologies have opened new avenues for the development of successful biopesticides and biofertilizers, which can further lead to the development of measures to eliminate pests in sustainable ways [93]. Different types of gene and genome mapping have been employed to understand different mechanisms of evolutionary inheritance in pests like plant hoppers [94], resistance mechanisms in diamondback moths [102], Hessian fly in wheat [95], planthopper resistance in rice crops [96], cotton resistant to white flies [87], and genome-wide association studies to understand cotton bolls infested by bollworms [88]. Pest risk analysis is the process of identifying appropriate phytosanitary measures to protect plant resources against new or emerging and regulated pests or plant products [97]. There is a need to explore more novel targets that can overcome problems like pesticide resistance [98], environmental impact [99], detrimental effects on non-target invertebrates [100], accumulation of pesticide residues in the food chain [101], and contamination of water supplies [100]. The transcriptome has been widely employed to investigate the molecular mechanisms underlying gene responses to insecticides and toxins [101].

They can be achieved in the following ways:

Figure 2 illustrates the different measures that can be undertaken for the management of pests of crops in a sustainable way from the time it is sown until the harvest.

8. CONCLUSION

Next-generation technology has proven to be a boon in various fields of healthcare and pesticide design. The advances in Bt cotton research had kept the pests under control for some time, but they eventually led to the emergence of secondary pests as the major pests. Even in Bt-cotton fields, various kinds of bollworms cause some of the highest yearly crop losses in Africa. As a result, transcriptome analysis is a research priority aimed not just at resolving difficulties caused by pests like *H. armigera* but also at a better understanding of significant insect resistance mechanisms in cash crops like cotton. There is a need to also understand other major pests of cotton and their resistance mechanisms. The identification of new and novel pest targets is the need of the hour. Transcriptome analyses for various applications like insecticide treatment, olfaction, and pheromone production processes in insects, as well as those involving herbivore stresses in the cotton crop, have revealed important information about the key novel genes and molecular signatures vital for the development of resistance mechanisms, which may further help to develop better ways for effective pests management. The use of a mix of high-throughput profiling techniques, bioinformatics tools, and ecological data will allow researchers to gain a full systems-level understanding of numerous elements of plant defense responses during insect attacks at multiple hierarchical levels. The use of molecular data and the employment of various bioinformatics approaches can further promote the development and deployment of sustainable strategies with the view of having a sustainable world.

9. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be authors as per the International Committee of Medical Journal Editors (ICMJE) requirements and guidelines.

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The authors report no financial or other conflicts of interest in this work.

12. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

13. DATA AVAILABILITY

All data generated and analyzed are included in this review article.

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